

What is claimed is:

1. A reference sample for maintaining prediction performance of an optical system used to measure an analyte or attribute in a representative measurement sample, wherein the representative measurement sample comprises a bodily tissue, bodily fluid or other biological sample containing the analyte or attribute, where the reference sample and the measurement sample absorb light at each of selected wavelengths in a manner to produce similarly shaped spectra over the wavelengths measured.

2. A reference sample as in claim 1, wherein the representative measurement sample includes multiple samples from multiple subjects.

3. A reference sample as in claim 2, wherein the reference sample has a spectral similarity ratio, when compared with the representative measurement sample spectra, of 30 or less when evaluated over the selected wavelengths measured.

4. The reference sample of claim 1, wherein the measurement sample is tissue and the selected wavelengths measured is a spectral range of  $4,200\text{ cm}^{-1}$  to  $7,200\text{ cm}^{-1}$ .

5. The reference sample of claim 1, wherein the measurement sample is tissue and the selected wavelengths measured is a spectral range of  $4,440\text{ cm}^{-1}$  to  $4,800\text{ cm}^{-1}$  and  $5,440\text{ cm}^{-1}$  to  $6,400\text{ cm}^{-1}$ .

6. The reference sample of claim 1, wherein the measurement sample is tissue, the analyte being measured is glucose and the selected wavelengths measured, in wavenumbers ( $\text{cm}^{-1}$ ), are selected from the group consisting of: 4196, 4227, 4273, 4281, 4304, 4320, 4335, 4366, 4389, 4436, 4451, 4459, 4497, 4528, 4559, 4613, 4690, 4775, 4829, 4860, 4883, 4922, 5014, 5091, 5176, 5230, 5269, 5299, 5315, 5338, 5369, 5392, 5454, 5469, 5477, 5515, 5585, 5623, 5662, 5701, 5731, 5755, 5785, 5809, 5839, 5893, 5924, 5947, 6001, 6094, 6163, 6187, 6287, 6318, 6349, 6449, 6472, 6557, 6595, 6673, 6696, 6935, 6973, 7004, 7043, 7066, 7205, and combinations thereof.

7. A reference sample as in claim 2, wherein the reference sample has a spectral similarity ratio, when compared with the representative measurement sample spectra, of 10 or less when evaluated over the selected wavelengths measured.

8. The reference sample of claim 3, wherein the measurement sample is tissue and the selected wavelengths measured is a spectral range of  $4,200 \text{ cm}^{-1}$  to  $7,200 \text{ cm}^{-1}$ .

9. The reference sample of claim 3, wherein the measurement sample is tissue and the selected wavelengths measured is a spectral range of  $4,440 \text{ cm}^{-1}$  to  $4,800 \text{ cm}^{-1}$  and  $5,440 \text{ cm}^{-1}$  to  $6,400 \text{ cm}^{-1}$ .

10. The reference sample of claim 3, wherein the measurement sample is tissue, the analyte being measured is glucose and the selected wavelengths measured, in

wavenumbers ( $\text{cm}^{-1}$ ), are selected from the group consisting of: 4196, 4227, 4273, 4281, 4304, 4320, 4335, 4366, 4389, 4436, 4451, 4459, 4497, 4528, 4559, 4613, 4690, 4775, 4829, 4860, 4883, 4922, 5014, 5091, 5176, 5230, 5269, 5299, 5315, 5338, 5369, 5392, 5454, 5469, 5477, 5515, 5585, 5623, 5662, 5701, 5731, 5755, 5785, 5809, 5839, 5893, 5924, 5947, 6001, 6094, 6163, 6187, 6287, 6318, 6349, 6449, 6472, 6557, 6595, 6673, 6696, 6935, 6973, 7004, 7043, 7066, 7205, and combinations thereof.

11. A reference sample as in claim 2, wherein the reference sample has a spectral similarity ratio, when compared with the representative measurement sample spectra, of 1 or less when evaluated over the selected wavelengths measured.

12. The reference sample of claim 11, wherein the measurement sample is tissue and the selected wavelengths measured is a spectral range of  $4,200 \text{ cm}^{-1}$  to  $7,200 \text{ cm}^{-1}$ .

13. The reference sample of claim 11, wherein the measurement sample is tissue and the selected wavelengths measured is a spectral range of  $4,440 \text{ cm}^{-1}$  to  $4,800 \text{ cm}^{-1}$  and  $5,440 \text{ cm}^{-1}$  to  $6,400 \text{ cm}^{-1}$ .

14. The reference sample of claim 11 wherein the measurement sample is tissue, the analyte being measured is glucose and the selected wavelengths measured, in wavenumbers ( $\text{cm}^{-1}$ ), are selected from the group consisting of: 4196, 4227, 4273, 4281, 4304, 4320, 4335, 4366, 4389, 4436, 4451, 4459, 4497, 4528, 4559, 4613, 4690, 4775,

4829, 4860, 4883, 4922, 5014, 5091, 5176, 5230, 5269, 5299, 5315, 5338, 5369, 5392, 5454, 5469, 5477, 5515, 5585, 5623, 5662, 5701, 5731, 5755, 5785, 5809, 5839, 5893, 5924, 5947, 6001, 6094, 6163, 6187, 6287, 6318, 6349, 6449, 6472, 6557, 6595, 6673, 6696, 6935, 6973, 7004, 7043, 7066, 7205, and combinations thereof.

15. A reference sample as in claim 2, wherein the reference sample has a regression weighted spectral similarity ratio, when compared to the representative measurement sample spectra, of 30 or less.

16. A reference sample as in claim 2, wherein the reference sample has a regression weighted spectral similarity ratio, when compared to the representative measurement sample spectra, of 10 or less.

17. A reference sample as in claim 2, wherein the reference sample has a regression weighted spectral similarity ratio, when compared to the representative measurement sample spectra, of 1 or less.

18. A reference sample as in claim 1, wherein the representative measurement sample is from a single subject.

19. A reference sample as in claim 18, wherein the reference sample has a spectral similarity ratio, when compared with the representative measurement sample spectra, of 1500 or less when evaluated over the selected wavelengths measured.

20. The reference sample of claim 19, wherein the measurement sample is tissue and the selected wavelengths measured is a spectral range of 4,200  $\text{cm}^{-1}$  to 7,200  $\text{cm}^{-1}$ .

21. The reference sample of claim 19, wherein the measurement sample is tissue and the selected wavelengths measured is a spectral range of 4,440  $\text{cm}^{-1}$  to 4,800  $\text{cm}^{-1}$  and 5,440  $\text{cm}^{-1}$  to 6,400  $\text{cm}^{-1}$ .

22. The reference sample of claim 19, wherein the measurement sample is tissue, the analyte being measured is glucose and the selected wavelengths measured, in wavenumbers ( $\text{cm}^{-1}$ ), are selected from the group consisting of: 4196, 4227, 4273, 4281, 4304, 4320, 4335, 4366, 4389, 4436, 4451, 4459, 4497, 4528, 4559, 4613, 4690, 4775, 4829, 4860, 4883, 4922, 5014, 5091, 5176, 5230, 5269, 5299, 5315, 5338, 5369, 5392, 5454, 5469, 5477, 5515, 5585, 5623, 5662, 5701, 5731, 5755, 5785, 5809, 5839, 5893, 5924, 5947, 6001, 6094, 6163, 6187, 6287, 6318, 6349, 6449, 6472, 6557, 6595, 6673, 6696, 6935, 6973, 7004, 7043, 7066, 7205, and combinations thereof.

23. A reference sample as in claim 18, wherein the reference sample has a spectral similarity ratio, when compared with the representative measurement sample spectra, of 1000 or less when evaluated over the selected wavelengths measured.

24. The reference sample of claim 23, wherein the measurement sample is tissue and the selected wavelengths measured is a spectral range of 4,200  $\text{cm}^{-1}$  to 7,200  $\text{cm}^{-1}$ .

25. The reference sample of claim 23, wherein the measurement sample is tissue and the selected wavelengths measured is a spectral range of 4,440  $\text{cm}^{-1}$  to 4,800  $\text{cm}^{-1}$  and 5,440  $\text{cm}^{-1}$  to 6,400  $\text{cm}^{-1}$ .

26. The reference sample of claim 23 wherein the measurement sample is tissue, the analyte being measured is glucose and the selected wavelengths measured, in wavenumbers ( $\text{cm}^{-1}$ ), are selected from the group consisting of: 4196, 4227, 4273, 4281, 4304, 4320, 4335, 4366, 4389, 4436, 4451, 4459, 4497, 4528, 4559, 4613, 4690, 4775, 4829, 4860, 4883, 4922, 5014, 5091, 5176, 5230, 5269, 5299, 5315, 5338, 5369, 5392, 5454, 5469, 5477, 5515, 5585, 5623, 5662, 5701, 5731, 5755, 5785, 5809, 5839, 5893, 5924, 5947, 6001, 6094, 6163, 6187, 6287, 6318, 6349, 6449, 6472, 6557, 6595, 6673, 6696, 6935, 6973, 7004, 7043, 7066, 7205, and combinations thereof.

27. A reference sample as in claim 18, wherein the reference sample has a spectral similarity ratio, when compared with the representative measurement sample spectra, of 1 or less when evaluated over the selected wavelengths measured.

28. The reference sample of claim 27, wherein the measurement sample is tissue and the selected wavelengths measured is a spectral range of 4,200  $\text{cm}^{-1}$  to 7,200  $\text{cm}^{-1}$ .

29. The reference sample of claim 27, wherein the measurement sample is tissue and the selected wavelengths measured is a spectral range of 4,440  $\text{cm}^{-1}$  to 4,800  $\text{cm}^{-1}$  and 5,440  $\text{cm}^{-1}$  to 6,400  $\text{cm}^{-1}$ .

30. The reference sample of claim 27 wherein the measurement sample is tissue, the analyte being measured is glucose and the selected wavelengths measured, in wavenumbers ( $\text{cm}^{-1}$ ), are selected from the group consisting of: 4196, 4227, 4273, 4281, 4304, 4320, 4335, 4366, 4389, 4436, 4451, 4459, 4497, 4528, 4559, 4613, 4690, 4775, 4829, 4860, 4883, 4922, 5014, 5091, 5176, 5230, 5269, 5299, 5315, 5338, 5369, 5392, 5454, 5469, 5477, 5515, 5585, 5623, 5662, 5701, 5731, 5755, 5785, 5809, 5839, 5893, 5924, 5947, 6001, 6094, 6163, 6187, 6287, 6318, 6349, 6449, 6472, 6557, 6595, 6673, 6696, 6935, 6973, 7004, 7043, 7066, 7205, and combinations thereof.

31. A reference sample as in claim 18, wherein the reference sample has a regression weighted spectral similarity ratio, when compared to the representative measurement sample spectra, of 4500 or less.

32. A reference sample as in claim 18, wherein the reference sample has a regression weighted spectral similarity ratio, when compared to the representative measurement sample spectra, of 1500 or less.

33. A reference sample as in claim 18, wherein the reference sample has a regression weighted spectral similarity ratio, when compared to the representative measurement sample spectra, of 1 or less.

34. A reference sample for maintaining prediction performance of an optical system used to measure an analyte or attribute in a representative measurement sample, wherein the representative measurement sample comprises a bodily tissue, bodily fluid or other biological sample containing the analyte or attribute, where the reference sample simulates the optical interaction between the measurement sample and the optical system.

35. A reference sample as in claim 34, wherein the reference sample has a spatial similarity, expressed in terms of standard deviation, of 0.079 or less.

36. A reference sample as in claim 34, wherein the reference sample has a spatial similarity, expressed in terms of standard deviation, of 0.052 or less.

37. A reference sample as in claim 34, wherein the reference sample has a spatial similarity, expressed in terms of standard deviation, of approximately 0.0.

38. A reference sample as in claim 34, wherein the reference sample has an angular similarity, expressed in terms of standard deviation, of 0.051 or less.

39. A reference sample as in claim 34, wherein the reference sample has an angular similarity, expressed in terms of standard deviation, of 0.036 or less.

40. A reference sample as in claim 34, wherein the reference sample has an angular similarity, expressed in terms of standard deviation, of approximately 0.0.

41. A optical measurement system used to measure an analyte or attribute in a biological system, the system comprising:

a spectrometer including an illumination source and a collection system;

a sampling system for performing measurements on tissue;

a measurement system for measuring multiple wavelengths in the range for  $4000\text{ cm}^{-1}$  to  $7500\text{ cm}^{-1}$ ;

a prediction process that uses multiple variables obtained from the measurement system; and

a reference sample wherein the primary optical absorber of the reference sample is water.

42. A reference sample as in claim 41, wherein the reference sample produces a spectrum composed of multiple optical pathlengths through water.

43. A reference sample as in claim 42, wherein the spectrum produced by the reference sample is not appropriately modeled by a water spectrum composed of only one pathlength of water.

44. A reference sample as in claim 42, wherein the spectrum produced by the reference sample is more appropriately modeled by water spectra representing multiple pathlengths of water.

45. The reference sample as in claim 42, wherein calculation of the multipath RMS error metric indicates the presence of multiple pathlengths of water.

46. The reference sample as in claim 42, wherein the multipath RMS error is greater than 0.1 absorbance units.

47. The reference sample as in claim 42, wherein the multipath RMS error is greater than 0.05 absorbance units.

48. A reference sample as in claim 41, wherein the reference sample produces an average optical pathlength of between 0.05 mm and 20 mm.

49. The reference sample as in claim 42, where the optical pathlength distribution produced by the reference sample is a warped gaussian.

50. A reference sample as in claim 42, wherein the multiple pathlengths of water are produced by using a diffuse reflecting surface.

51. A reference sample as in claim 42, wherein the multiple pathlengths of water are produced by placement of scattering media in the water.

52. A reference sample as in claim 42, wherein the multiple pathlengths of water are produced by creating physically different pathlengths through water.

53. A reference sample as in claim 42, further comprising a concentration of an analyte, said analyte being the same as that which is to be measured by the optical spectroscopy system.

54. A reference sample for maintaining prediction performance of an optical system used to measure an analyte or attribute in a representative measurement sample, wherein the representative measurement sample comprises a bodily tissue, bodily fluid or other biological sample containing the analyte or attribute, where the reference sample has the same primary optical absorber as the measurement sample.

55. A reference sample as in claim 54, wherein the representative measurement sample contains first and second primary constituents, and wherein the reference sample contains the same first and second primary constituents.

56. A reference sample as in claim 55, wherein the second primary constituent comprises protein.

57. A reference sample as in claim 55, wherein the second primary constituent comprises lipid.

58. A reference sample as in claim 55, wherein the second primary constituent comprises a organic polymer.

59. A reference sample as in claim 54, wherein the measurement sample is tissue and the portion of the reference sample that is optically sampled contains less than 80% water by volume.

60. A reference sample for maintaining prediction performance of an optical system used to measure an analyte or attribute in a representative measurement sample, wherein the representative measurement sample comprises a bodily tissue, bodily fluid or other biological sample containing the analyte or attribute, with the reference sample producing a reference spectrum that is optically similar to the representative measurement sample.

61. A reference sample as in claim 60, wherein the reference sample includes:  
an optically transparent layer;  
a diffusing layer; and

a constituent layer disposed between the optically transparent layer and the diffusing layer.

62. A reference sample as in claim 61, wherein the representative measurement sample contains a primary constituent, and wherein the constituent layer contains the same primary constituent.

63. A reference sample as in claim 62, wherein the constituent layer contains water.

64. A reference sample as in claim 62, wherein the constituent layer contains protein.

65. A reference sample as in claim 62, wherein the constituent layer contains lipid.

66. A reference sample as in claim 61, wherein the diffusing layer is cone shaped.

67. A reference sample as in claim 61, wherein the optically transparent layer, the constituent layer, and the diffusing layer are cone shaped.

68. A reference sample as in claim 61, wherein the diffusing layer is non-planar.

69. A reference sample as in claim 62, wherein the optically transparent layer is flat.

70. A reference sample as in claim 60, wherein the reference sample includes:  
a container that is at least partially optically transparent; and  
a scattering solution in the container.

71. A reference sample as in claim 70, wherein the reference sample further includes a stirring mechanism for stirring the scattering solution.

72. A reference sample as in claim 70, wherein the scattering solution comprises reflective beads disposed in a constituent.

73. A reference sample as in claim 60, wherein the reference sample includes:  
a first optical splitting layer;  
a reflective layer; and  
a first constituent layer disposed between the first optical splitting layer and the reflective layer.

74. A reference sample as in claim 73, wherein the representative measurement sample contains a primary constituent, and wherein the first constituent layer contains the same primary constituent.

75. A reference sample as in claim 74, wherein the constituent layer contains water.

76. A reference sample as in claim 73, wherein the reference sample further includes:

a second optical splitting layer; and

a second constituent layer disposed between the first optical splitting layer and the second optical splitting layer.

77. A reference sample as in claim 55, wherein the reference sample includes:

a container that is at least partially optically transparent;

a constituent disposed in the container; and

a spacer disposed in the container.

78. A reference sample as in claim 77, wherein the representative measurement sample contains a primary constituent, and wherein the constituent disposed in the container comprises the same primary constituent.

79. A reference sample as in claim 78, wherein the constituent disposed in the container comprises water.

80. A reference sample as in claim 77, wherein multiple spacers are disposed in the container.

81. A reference sample as in claim 60, wherein the reference sample includes:  
an optically transparent layer;  
a diffuse reflective layer disposed a distance from the optically transparent layer;  
and  
a constituent layer disposed between the optically transparent layer and the diffuse reflective layer.

82. A reference sample as in claim 81, wherein the representative measurement sample contains a primary constituent, and wherein the constituent layer comprises the same primary constituent.

83. A reference sample as in claim 81, wherein the constituent layer comprises water.

84. A reference layer as in claim 81, wherein the diffuse reflective layer is movable relative to the optically transparent layer to change the distance therebetween.

85. A reference sample as in claim 60, wherein the reference sample includes:  
a animal based bodily constituent.

86. A reference sample as in claim 85, wherein the animal based bodily  
constituent comprises animal bodily tissue.

87. A reference sample as in claim 85, wherein the animal based bodily  
constituent comprises animal bodily fluid.

88. A reference sample as in claim 60, wherein the reference sample includes:  
a gel matrix;  
scattering media; and  
water.

89. A reference sample as in claim 88, wherein the gel matrix is placed in  
direct contact with the optical sampling device.

90. A reference sample as in claim 88, wherein the gel matrix is contained in a  
container that enables optical sampling.

91. An optical system used to measure an analyte or attribute in a sample of  
interest, wherein the sample comprises a bodily tissue, bodily fluid or other biological  
sample containing the analyte or attribute, the optical spectroscopy system comprising:

a spectrometer including an illumination source and a collection system; and

a reference sample producing a reference sample spectrum that has spectral similarity to a representative measurement sample spectrum, the reference sample optically sampled by reflectance sampling.

92. An optical spectroscopy system as in claim 91, wherein the reference sample spectrum has a spectral similarity ratio, when compared to the representative measurement sample spectra, of 30 or less.

93. An optical spectroscopy system as in claim 91, wherein the reference sample spectrum has a regression weighted spectral similarity ratio, when compared to the representative measurement sample spectra, of 30 or less.

94. An optical spectroscopy system as in claim 91, wherein the sample of interest contains a primary constituent, and wherein the reference sample contains the same primary constituent.

95. An optical spectroscopy system as in claim 94, wherein the primary constituent comprises water.

96. A method for producing accurate optical measurement predictions by using an optical system to measure an analyte or attribute in a test sample of interest, wherein the test sample comprises a bodily tissue, bodily fluid or other biological sample

containing the analyte or attribute, the test sample producing a test sample spectrum when subjected to spectrographic analysis, the method comprising the steps of:

providing a spectrometer including an illumination source and a collection system;

providing a reference sample producing a reference spectrum that is similar to a representative measurement sample spectrum;

optically coupling the reference sample to the illumination source of the optical spectrometer;

irradiating the reference sample with multiple wavelengths of radiation from the illumination source;

collecting radiation that is not absorbed by the reference sample with the collection system;

determining intensities of the non-absorbed radiation at the multiple wavelengths to generate a reference spectrum; and

modifying the spectral data from the test sample based on the reference sample background spectrum and deriving the analyte or attribute measurement with the corrected spectral data.

97. The method of claim 96, wherein correcting the spectral data from the test sample includes subtracting the reference sample spectrum from the test sample spectrum.

98. The method of claim 96, wherein correcting the spectral data from the test sample includes taking the ratio of the test sample spectrum relative to the reference sample spectrum.

99. A method of establishing and/or maintaining a multivariate calibration model in an optical system used to measure an analyte or attribute in a test sample of interest, wherein the test sample comprises a bodily tissue, bodily fluid or other biological sample containing the analyte or attribute, the method comprising the steps of:

providing a reference sample having a reference spectrum that is spectroscopically similar to the test sample spectrum;

obtaining a reference sample spectrum from the reference sample using the spectroscopy system; and

using the reference sample spectrum to maintain the prediction performance of the optical measurement system.

100. A method as in claim 99, further comprising the steps of:

obtaining a new test sample spectrum from a new test sample using the spectroscopy system; and

predicting the analyte or attribute of the test sample utilizing the calibration model and the new test spectrum.

101. A method as in claim 99, wherein multiple reference spectra are obtained over a period of time.

102. A method as in claim 101, wherein the multiple reference spectra are time-averaged just prior to obtaining the test spectrum.

103. A reference sample for maintaining prediction performance of an optical system used to measure an analyte or attribute in a test sample of interest, wherein the test sample comprises a bodily tissue, bodily fluid or other biological sample containing the analyte or attribute, with the reference sample producing a reference sample spectrum that is similar to the test sample spectrum, the reference sample including a transmissive optical interface and an optical sampling compartment, the optical sampling compartment containing water and a diffusely reflective or scattering media.

104. A reference sample as in claim 103, wherein the reference sample spectrum has a spectral similarity ratio, when compared to a representative measurement sample spectra, of 30 or less.

105. A reference sample as in claim 103, wherein the reference sample spectrum has a regression weighted spectral similarity ratio, when compared to a representative measurement sample spectra, of 30 or less.

106. A reference sample as in claim 103, wherein the reference sample has a spatial similarity, expressed in terms of standard deviation, of 0.079 or less.

107. A reference sample as in claim 103, wherein the reference sample has an angular similarity, expressed in terms of standard deviation, of 0.051 or less.

108. A reference sample for maintaining prediction performance of an optical system used to measure an analyte or attribute in a representative measurement sample, wherein the representative measurement sample comprises a bodily tissue, bodily fluid or other biological sample containing the analyte or attribute, where the reference sample has a different primary optical absorber when compared to the measurement sample.

109. The reference sample of claim 108, wherein the spectral similarity is achieved by using at least one of the following: optical filters, absorbance filters, interference filters, or reflectance material.